Monocular microsaccades are visual-task related

Josselin Gautier
Anglia Vision Research, Department of Vision and Hearing Sciences, Anglia Ruskin University, Cambridge, UK
Davalor Research Center, Universitat Politècnica de Catalunya, Barcelona, Spain

Harold E. Bedell
College of Optometry, University of Houston, Houston, TX, USA

John Siderov
Anglia Vision Research, Department of Vision and Hearing Sciences, Anglia Ruskin University, Cambridge, UK

Sarah J. Waugh
Anglia Vision Research, Department of Vision and Hearing Sciences, Anglia Ruskin University, Cambridge, UK

During visual fixation, we constantly move our eyes. These microscopic eye movements are composed of tremor, drift, and microsaccades. Early studies concluded that microsaccades, like larger saccades, are binocular and conjugate, as expected from Hering's law of equal innervation. Here, we document the existence of *monocular* microsaccades during both fixation and a discrimination task, reporting the location of the gap in a foveal, low-contrast letter C. Monocular microsaccades differ in frequency, amplitude, and peak velocity from binocular microsaccades. Our analyses show that these differences are robust to different velocity and duration criteria that have been used previously to identify microsaccades. Also, the frequency of monocular microsaccades differs systematically according to the task: monocular microsaccades occur more frequently during fixation than discrimination, the opposite of their binocular equivalents. However, during discrimination, monocular microsaccades occur more often around the discrimination threshold, particularly for each subject's dominant eye and in case of successful discrimination. We suggest that monocular microsaccades play a functional role in the production of fine corrections of eye position and vergence during demanding visual tasks.

Introduction

The occurrence of microsaccades during fixation has been reported since 1907 (Dodge, 1907). Thereafter, multiple studies using different recording techniques concluded that microsaccades are binocular coordinated movements with similar timing, directions (conjugacy), and velocities in the two eyes (Ditchburn & Ginsborg, 1953; Krauskopf, Cornsweet, & Riggs, 1960; Schulz, 1984; St. Cyr & Fender, 1969; Yarbus, 1967). Zuber, Stark, and Cook (1965) demonstrated that saccades, microsaccades, and corrective saccades (see next paragraph) lie on a single linear peak velocity versus amplitude relationship, the so-called main sequence, and deduced that these movements result from a common physiological mechanism. Boyce (1967) observed that the amplitude distribution of microsaccades during monocular fixation exhibits a cut-off around 12′ with a few outliers around 20′. A review of the fixation data from 14 experiments by Ditchburn and Foley-Fisher (1967) confirmed these results and indicated a median microsaccadic amplitude during fixation of 4′–5′. More recent studies reported a larger mean microsaccade size, between 13.7′ and 38.4′ (see the review by Martinez-Conde, Macknik, Troncoso, & Hubel, 2009). The disagreement about the amplitude characteristics of microsaccades

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(Collewijn & Kowler, 2008) might be traced in part to different levels of subject expertise (Cherici, Kuang, Poletti, & Rucci, 2012; Martinez-Conde et al., 2009) and recording conditions, considering that recordings were mainly made with experienced subjects using the optical lever technique in the 1950s and 1960s, whereas recent studies of fixation used video-based eye tracking systems on naïve participants. Cherici et al. (2012) also emphasize that previous studies typically assumed that the distribution of gaze positions during fixation is normal, an assumption that does not hold. It is also acknowledged that although the distinction between microsaccades and saccades based on their size ($<1°$) is arbitrary, this distinction is practical in that it captures approximately 90% of the nonvoluntary fixational saccades (Martinez-Conde et al., 2009).

In contrast to the classical viewpoint that fixation saccades are binocular and conjugate, Engbert & Kliegl (2003a) identified a significant number of *monocular* microsaccades during fixation, which were reported subsequently to be inefficient in counteracting visual fading (Martinez-Conde, Macknik, Troncoso, & Dyar, 2006). Several investigators reported that binocular microsaccades occur mainly in the horizontal direction, while monocular microsaccades occur in both the horizontal and vertical directions (Engbert & Kliegl, 2003a; Hermens & Walker, 2010; Kloke, Jaschinski, & Jainta, 2009). Disconjugate microsaccades have also been observed and measured neurophysiologically in monkeys (Horn & Cullen, 2012), in whom a significant proportion (approx. 10%) could potentially be monocular microsaccades (see their figure 1F). Despite the suggestions that monocular microsaccades might have a different origin and should be analyzed separately from their binocular counterparts (Hermens & Walker, 2010), the conditions that favor monocular microsaccades, their kinematic properties, and their potential role in vision has not been explored. In this work, we aim to assess the occurrence and characteristics of monocular microsaccades during two visual tasks involving either involuntary alone, or both involuntary and voluntary fixational eye movements, and examine how monocular microsaccades differ from their previously reported binocular counterparts.

Small monocular corrective movements that occur following saccades, presumably because of neurological control signal reversals (Bahill, Clark, & Stark, 1975) and especially microsaccades, were reported in the literature as dynamic overshoots (Abadi, Scallan, & Clement, 2000; Kapoula, Robinson, & Hain, 1986) and ringing (Kimmel, Mammo, & Newsome, 2012). Unlike dynamic overshoots, the monocular saccades we observe do not follow immediately after microsaccades, even if—as we will suggest—they also may play a corrective role.

In the recent booming literature about fixational eye movements, there is common agreement to base the detection of microsaccades on three criteria: their peak velocity, a minimum duration, and temporal coordination between the two eyes (Engbert & Kliegl, 2003b). First, the peak eye-movement velocity should exceed a velocity threshold $\eta_{x,y}$ calculated typically as 4 to 6 standard deviations above the median eye velocity (see equations 2 and 3 in Engbert & Mergenthaler, 2006). Second, the movement must exceed a minimal duration to ensure that very brief high-velocity events, such as dynamic overshoots or instances of velocity noise, are not identified as a separate microsaccade. Although the minimum duration is not always mentioned and is sampling-frequency dependent, a representative range of values is 6–12 ms (Engbert & Kliegl, 2003b, 2004; Engbert & Mergenthaler, 2006). Finally, a criterion of a temporal overlap of microsaccades made by both eyes has been used to "reduce noise in the detection procedure" according to Engbert and Kliegl (2003b, p. 1037). Thus, in the literature spanning the last decade, monocular microsaccades detected in only one eye that could have been detected through binocular recordings were indeed excluded (Engbert & Kliegl, 2003b; Martinez-Conde et al., 2009).

Methods

Observers

Ten subjects (five men, five women; age range 21–35 years) with normal or corrected-to-normal visual acuity participated in the study. The data for two subjects were discarded due to spurious signals, probably from unavoidable reflections from their spectacles. With the exception of author JG, the subjects were naïve about the purpose of the experiments. Procedures were approved by the ethics board of the Anglia Ruskin University, Cambridge, UK, in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and written informed consent was obtained from all observers.

Apparatus

Stimuli were generated in Matlab (MathWorks, Natick, MA), using Psychophysics Toolbox extension and displayed on a 21 in. CRT screen (NEC Multisync 2141SB) with spatial resolution of 1600×1200 pixels, a refresh rate of 85 Hz, and a background luminance of 100 cd/m^2. The stimuli were displayed at a viewing

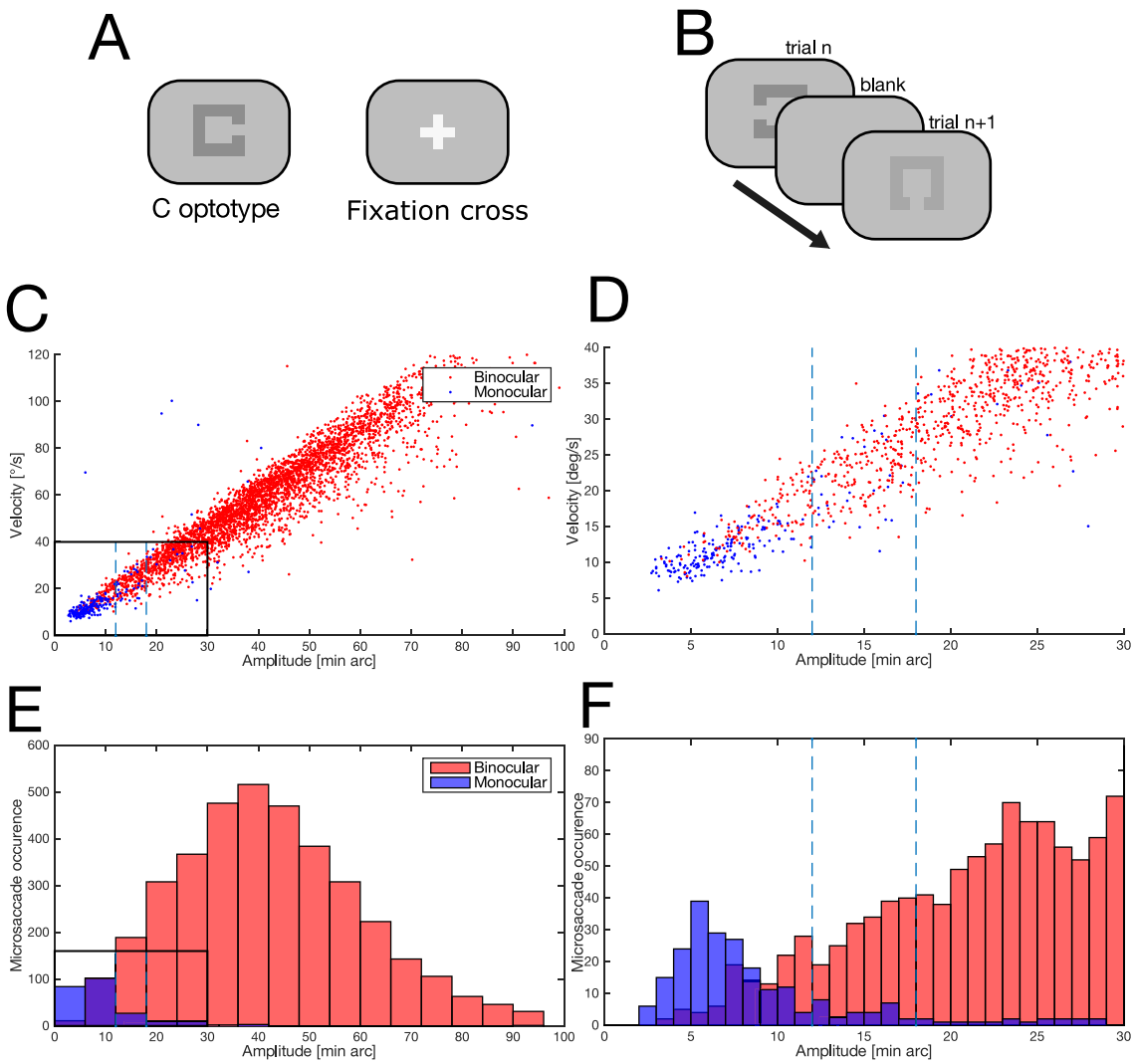

Figure 1. Discrimination versus fixation conditions. (A) Illustration of the stimuli for discrimination (left) and fixation (right). (B) In both conditions, the stimuli were alternated with a blank screen that was displayed until the subject pressed a response button that initiated the next trial. As shown here, the orientation and contrast of the discrimination stimulus varied randomly from trial to trial. (C) The main sequence diagram pooled for eight subjects for monocular (left eye only; shown in blue for clarity) and binocular microsaccades (red) during the discrimination task. (D) A zoomed-in view of the lower left section (box in panel C) of the main sequence plot. (E) The corresponding amplitude distribution of monocular (blue) and binocular (red) microsaccades for all participants, displayed with transparency (purple when bars overlay). (F) A zoomed-in view (box in panel E) of the small-amplitude region of the microsaccade amplitude distribution. Vertical dashed lines in panels C, D, and F are positioned at 12′ and 18′.

distance of 1.5 m in a silent room. At this viewing distance, each pixel subtended 0.0095°.

The stimuli were viewed binocularly while eye movements were recorded with an EyeLink 1000 (SR Research, Ottawa, ON, Canada) at a sampling rate of 500 Hz. According to the manufacturer, the eye tracker has a typical spatial accuracy between 0.25 and 0.5° (optimal = 0.1°), a noise-limited instrument spatial resolution of 0.01°, and a microsaccade resolution of 0.05°. To optimize accuracy and resolution of the recorded data, a bite bar kept the subjects' head as still as possible during the experiment.

Stimuli and procedure

The present study was designed originally to evaluate the fixational eye movements made during the discrimination of the orientation of a square letter C presented in one of the four cardinal orientations. Inspection of eye-movement records indicated that a significant proportion of the identified microsaccades met our velocity and duration criteria only in one eye. Thus, we compared the microsaccadic movements made while the head was restrained rigidly using a bitebar during two different conditions: discrimination versus fixation.

In the discrimination condition, participants reported in which of four orientations a 40′ diameter square low contrast C was presented (Figure 1A). Although the size of the C was well above the foveal resolution threshold, the discrimination task was made challenging by displaying the C at one of five low contrast levels on a lighter gray background (Figure 1B). The contrast levels were chosen to ensure that discrimination performance varied from chance to nearly 100% correct (see Appendix A). A total of 100 2-s trials were presented per session, during which horizontal and vertical eye position was recorded binocularly using the EyeLink 1000 eye tracker. Because the diameter of the C was 40′, all observed saccades were expected to be "microsaccades" according to the current definition (Engbert & Kliegl, 2003b). After each trial, the observer pressed one of four buttons to report the orientation of the C, guessing if necessary. For the fixation condition, participants were asked to maintain fixation at the center of a clearly visible bright cross (Weber contrast = +9.8%, each arm = $10' \times 2'$) during a series of 100 2-s presentations. All subjects performed four sessions of 100 trials each, two sessions of discrimination, and two sessions of fixation.

Detection of microsaccades

Horizontal and vertical changes between successive eye-position samples were combined to calculate the Pythagorean eye velocity, separately for each eye. Microsaccades were defined when the absolute value of the velocity signal was at least 6 *SD* ($\lambda = 6$) greater than the median eye speed during a 2-s trial for a minimum of six velocity samples ($\tau = 12$ ms). This duration criterion leads to a conservative number of monocular microsaccades. A smaller minimum duration (as often used in the literature; Engbert & Kliegl, 2003b; Poletti, Listorti, & Rucci, 2013; Watanabe, Matsuo, Zha, Munoz, & Kobayashi, 2013) produced greater absolute proportions of monocular microsaccades, similar to the value of 40% in the literature (see Appendix D1; Engbert & Kliegl, 2003a; Hermens & Walker, 2010; Kloke et al., 2009). Nevertheless, these previous values are likely to be overestimated because of recording noise. In the current study, monocular and binocular microsaccades were identified, respectively, if the velocity signals for only one eye or both eyes (i.e., with a temporal overlap) met the criteria for velocity and duration.

Nondetection of dynamic overshoot, ringing, and pupil-related artifacts

Ringing has been observed to occur primarily when using optical (video) eye trackers, due to the physiological elasticity properties of the eye tissues (Kimmel et al., 2012) or when using dual-Purkinje eye trackers due to elasticity of the lens zonules (Tabernero & Artal, 2014). Ringing could lead to the erroneous identification of artifactual microsaccades when using only the criteria of minimum eye velocity and movement duration. In the literature, an additional criterion of a 20 ms minimum intersaccadic interval has been used (Martinez-Conde et al., 2009; Møller, Laursen, Tygesen, & Sjølie, 2002) to overcome this problem. We chose to fuse as a single microsaccade two microsaccades separated by less than 10 ms in order to detect as many microsaccades as possible while excluding ringing and dynamic overshoot as potential artifacts.

Choe, Blake, and Lee (2016) reported a possible interaction between recorded changes in pupil area and the measured gaze position during fixation, for both "large" (approx. 6 min) and "short" time scales (13.2 s), but indicated a "negligible influence of the correction procedure on microsaccade detection" (p. 9). Below in Appendix C, we report consistently low correlations between the change of pupil size and change of horizontal, vertical, and Pythagorean eye positions (and also velocities) during identified monocular microsaccades.

Results

The first part of the results compares the kinematic properties of monocular and binocular microsaccades. The second part presents statistical evidence for an effect of the task on the monocular microsaccades rate. The third part reveals how monocular microsaccade production is modulated by visibility during the discrimination task.

The main sequence diagram of identified microsaccades (Figure 1C) indicates that monocular and binocular microsaccades generally follow the same peak velocity versus amplitude relationship (bisquare robust linear regression; Dumouchel & O'Brien, 1991; $R^2 = 0.88$ [0.6 using nonrobust regression], $R^2 = 0.97$ [0.69]), in agreement with previous literature (Bahill et al., 1975; Zuber et al., 1965) and suggesting that both are driven by the same type of oculomotor command. However, the amplitude distributions of monocular and binocular microsaccades (Figure 1E, F; see also Figure 2A) show a marked difference, with the size of monocular microsaccades exhibiting a peak at very small amplitudes and rarely exceeding 18′ (mean 10.2′, median 7.2′). Binocular microsaccade amplitudes on the other hand are approximately normally distributed, with a peak around 40′ (mean 42.8′, median 41.1′) during the discrimination task. This dramatic difference in the amplitude distributions of monocular and

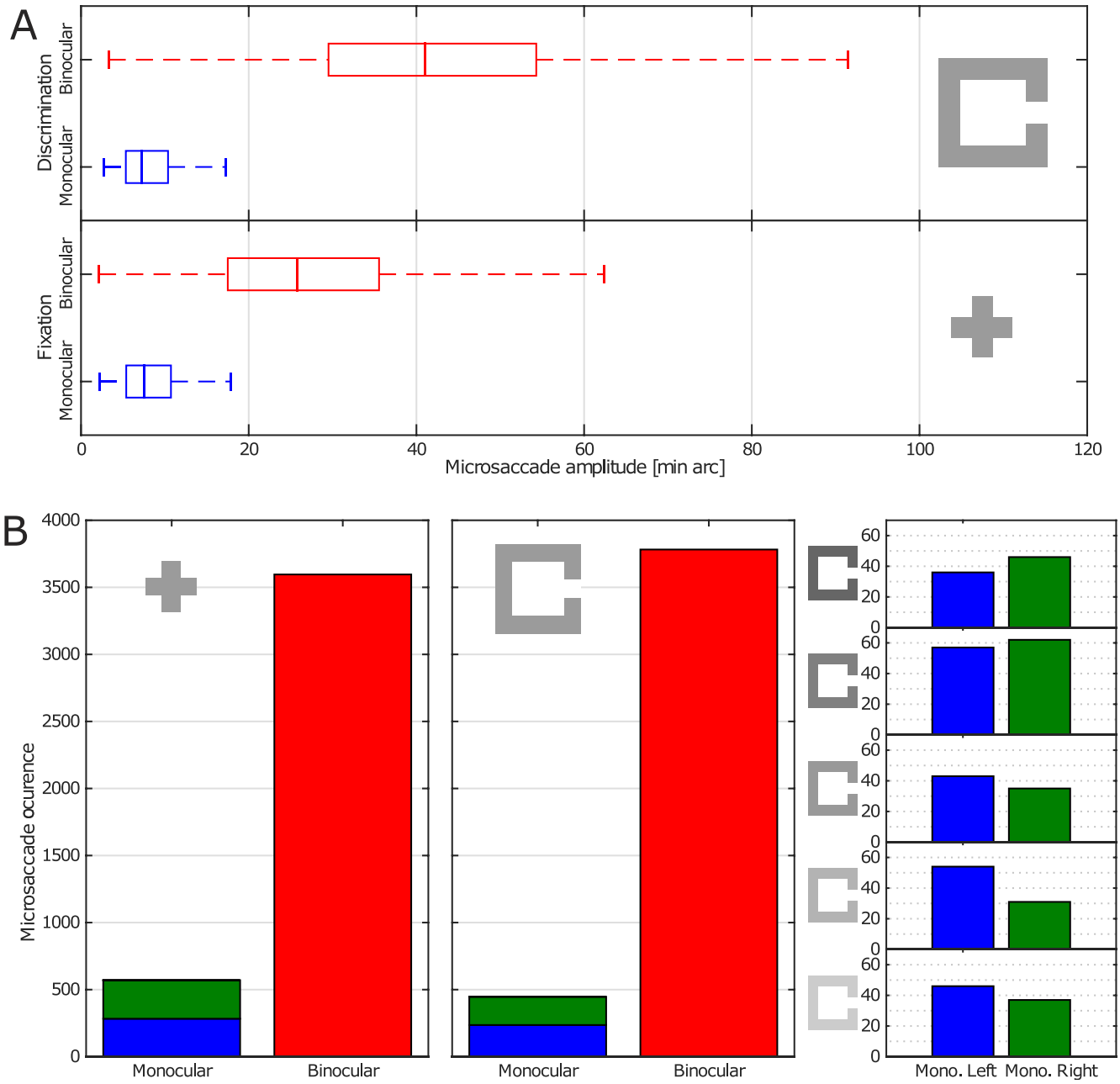

Figure. 2. Comparison of monocular and binocular microsaccade characteristics for two different conditions. (A) Boxplots of the amplitudes of the binocular (red) and monocular left-eye (blue) microsaccades, pooled across eight observers, during the discrimination (top) and fixation (bottom) conditions. Each box delineates the first and third quartile, whiskers extend from the ninth to the 91st percentile, and the vertical line inside the box refers to the median. (B) Corresponding occurrences of monocular left-eye (blue), right-eye (green), and binocular (red) microsaccades for the two conditions, pooled across observers. Bottom right panel: variation of monocular microsaccade occurrence with stimulus visibility for the discrimination condition.

binocular microsaccades occurred also during the fixation condition (Figure 2A).

Interestingly, the subjects' task influences the amplitude and frequency of monocular and binocular microsaccades (Figure 2A, B). During discrimination, the frequency of binocular microsaccades is overall (i.e., pooled across all visibility steps) greater than during the fixation condition, and the binocular microsaccades during discrimination also are larger in amplitude, for all subjects. An increased frequency of binocular microsaccades during discrimination is in agreement with previous studies (Ko, Poletti, & Rucci, 2010; Poletti et al., 2013; Rucci, Iovin, Poletti, & Santini, 2007). The increase in binocular microsaccade amplitude is likely to result from the inclusion of voluntary fixation shifts that the subjects made to

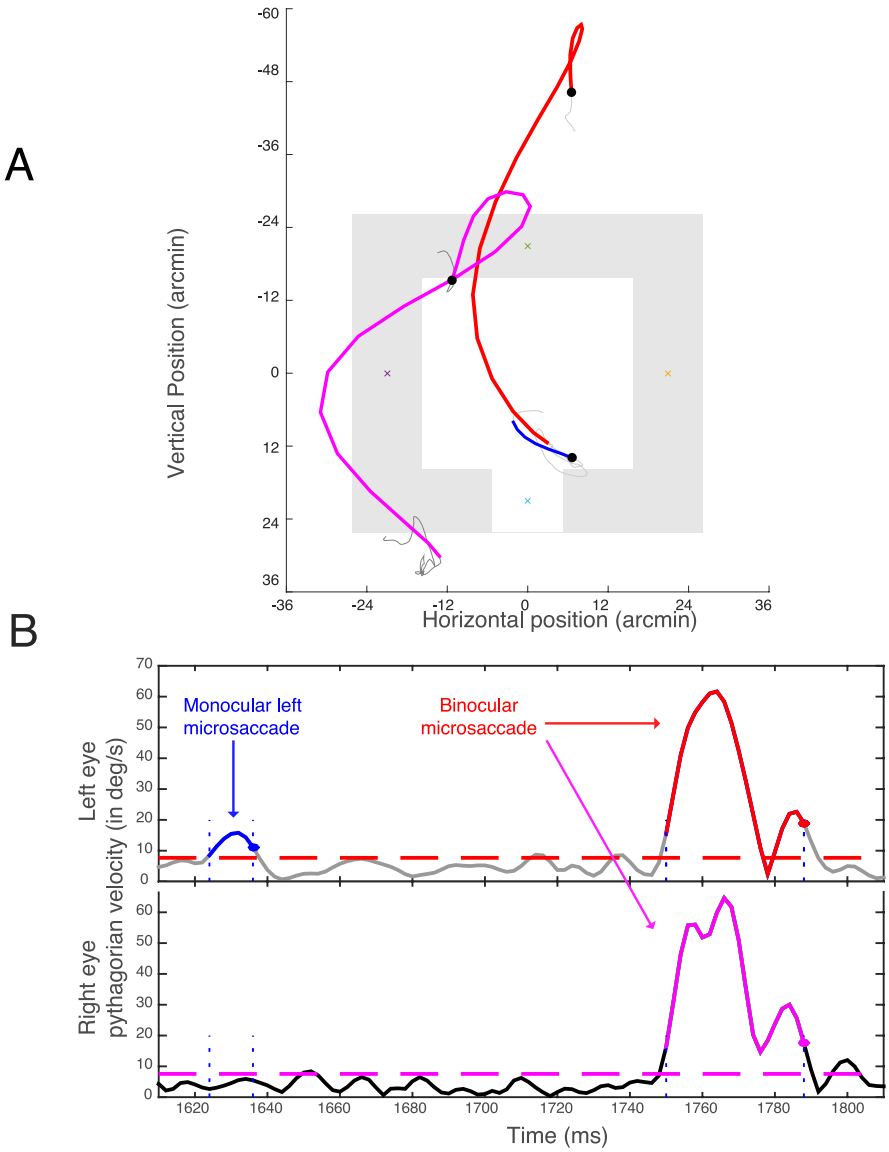

Figure 3. Microsaccade trajectories and corresponding velocity traces for both eyes. (A) Eye-position trajectories for one subject during 1610–1820 ms of one discrimination trial superimposed on the C target for illustration. Drifts of the left and right eye are shown in light and dark grey, respectively. A detected monocular microsaccade of the left eye is represented in blue, and a binocular microsaccade is shown as red in the left eye's trace and magenta in the right eye's trace. Dark points represent each microsaccade landing position. (B) Pythagorean velocity of the left (top) and right (bottom) eyes for this time interval illustrating the monocular microsaccade made by the left eye ($t = 1620$ ms), followed by a larger binocular microsaccade ($t = 1750$ ms). The small velocity peak in the right eye at $t \approx 1800$ ms is not categorized as a monocular microsaccade because it does not meet the 12-ms duration criterion.

inspect the different possible gap positions in the C while making the discrimination.

In contrast, the frequency of monocular microsaccades during the visual-discrimination task is overall slightly reduced, compared to fixation (from 283 [7.3%] to 236 [5.8%] for monocular microsaccades by the left eye and from 287 [7.3%] to 211 [5.3%] for monocular microsaccades by the right eye). This reduction is significant for both eyes, $\chi^2(1) = 8.5$, $p < 0.05$, $\chi^2(1) = 23.2$, $p < 0.001$. The same trends are observed if different velocity and duration criteria are applied to define microsaccades ($\lambda = 4$ or 5 and $\tau = 8$ or 10 ms).

To assess the possible influence of velocity noise on the identification of monocular microsaccades, we performed three analyses. First, we validated that the addition of independent plausible velocity noise (extracted from consecutive trials) does not lead to an overdetection of monocular microsaccades (i.e., false positives; see Appendix B). This outcome is attributable to the conservative but robust velocity threshold calculated as 6 times the standard deviation of the

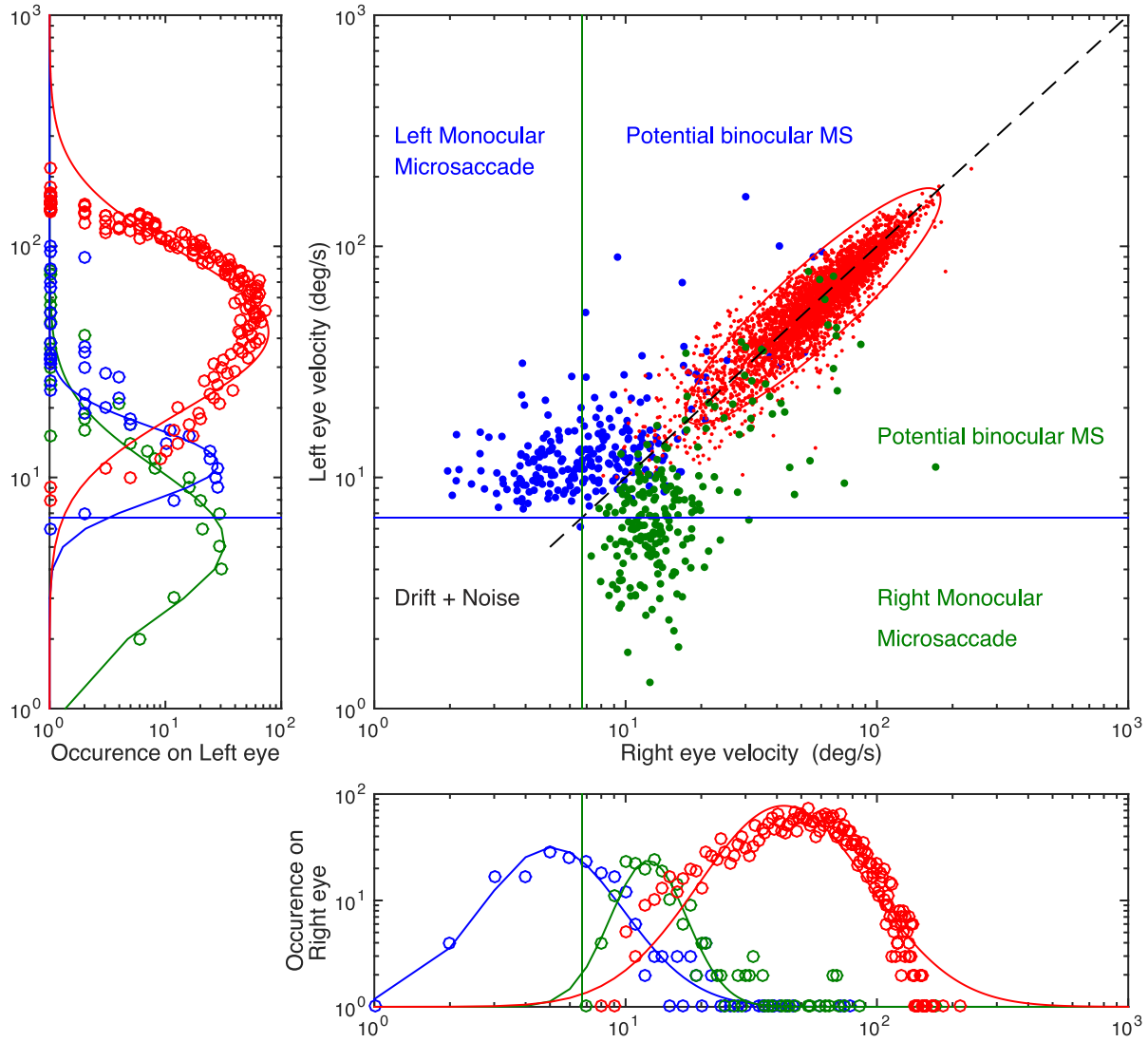

Figure 4. Analysis of the peak velocity ratio between the two eyes during monocular and binocular microsaccades. Scatter plot of left versus right eye peak velocities, for microsaccades detected in the left eye only (blue), the right eye only (green), and for both eyes synchronously (red). The horizontal and vertical blue and green lines correspond to the threshold of drift and noise defined as the mean +2 *SD*. The red ellipse represents the 95% confidence interval for the binocular microsaccade velocities. The left and bottom panels illustrate fits to the distributions of peak velocity for each eye when that eye performs a monocular microsaccade (left eye, blue; right eye, green), when the other eye performs a monocular microsaccade, and when both eyes perform a binocular microsaccade (red).

median eye velocity. Second, we quantified the median values of the velocity thresholds as 7.94°/s ($SD = 1.36$°/s) and 9.09°/s ($SD = 2.74$°/s) for the left and right eyes, respectively. On the other hand, the median peak velocities of monocular microsaccades are 12.26°/s and 13.95°/s for the left and right eye (i.e., respectively, 3 and 1.8 *SD*s above the median velocity threshold). Hence, a majority of the identified monocular microsaccades for each eye is substantially more than 6 *SD*s above the velocity median, which already represents a conservative criterion for microsaccade detection. Third, we analyzed the distribution of the between-eye peak-velocity ratios during both binocular and monocular microsaccades (Figure 4). During monocular microsaccades, this ratio is defined using the peak velocity of the eye that initiated the microsaccade compared to the peak velocity of the contralateral eye during the same time interval. A two-sample Kolmogorov-Smirnov test revealed that the distributions of the log microsaccade peak velocity ratios are significantly different between the two tasks (|most extreme difference| = 3.5%, $t = 1.60$, $N = 8{,}395$, $p < 0.05$), indicating that the larger amplitudes and peak velocities of the microsaccades in the discrimination condition can not account for lower frequency of monocular microsaccades in this task. The lower rate of

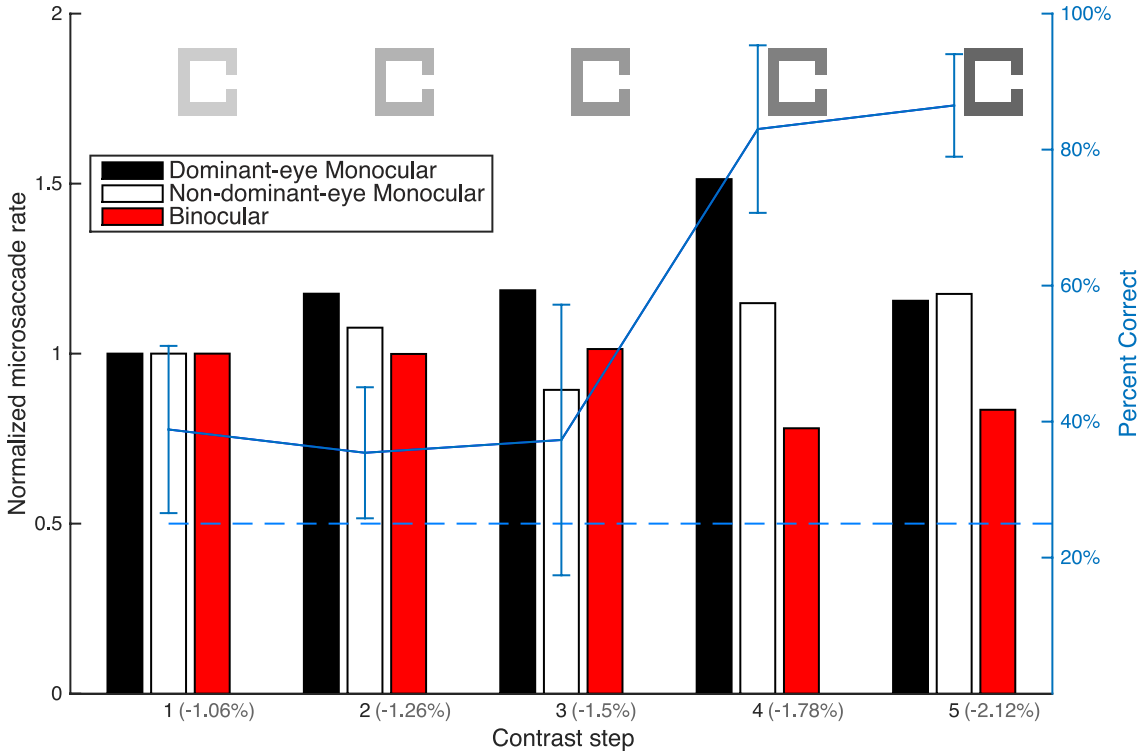

Figure 5. Analysis of microsaccade frequency versus discrimination performance. Average rate of monocular dominant-eye (black), nondominant-eye (white), and binocular (red) microsaccades, each normalized to unity at the lowest contrast visibility step in the discrimination condition (1). The background plot illustrates the percentage of correct orientation discrimination as a function of letter contrast, averaged for all subjects, with ±1 standard deviation represented as the shaded area. The Weibull function fitted to the discrimination responses is superimposed in blue, with a threshold at 62.5%. Note that chance performance corresponds to 25% correct (dashed blue line).

monocular microsaccades during discrimination therefore could indicate more conjugate control of the microsaccades made during this more attentionally demanding task, or might indicate a specific, independent role for monocular microsaccades during discrimination. As we will see, the rate of monocular microsaccades varies significantly with the visibility of the target to discriminate.

We hypothesized that there might be an effect of eye dominance on the production of monocular microsaccades. Ocular dominance was measured on each subject with two standard sighting dominance assessments, the hole-in-the-card test (Dolman method) and the point-a-finger test (Porta test; Li et al., 2010). A higher rate of monocular microsaccades is associated with the dominant eye during discrimination (consistent on 12/16 sessions, phi = 0.5 $p < 0.05$). In addition, χ^2 tests indicate that monocular microsaccades occur significantly more often in the dominant than the nondominant eye during the discrimination task, $\chi^2(1) = 21.30$, $p < 0.001$, and, to a lesser extent, during the fixation task, $\chi^2(1) = 6.19$, $p < 0.05$. During discrimination, monocular microsaccades occurred more often in the dominant eye on successful trials (above chance levels, 61% vs. 39% of all monocular microsaccades, $\chi^2(1) = 20.15$, $p < 0.001$) but not on unsuccessful trials (52% vs. 48%, $\chi^2(1) = 0.26$, $p = 0.61$). These outcomes suggest a specific role for monocular microsaccades during discrimination.

To elucidate the role of the small monocular microsaccades during discrimination we compared their frequencies in the subjects' dominant and nondominant eye to the frequencies of binocular microsaccades for the different contrast steps of C visibility (Figure 5). On one hand, the frequency of *binocular* microsaccades reduces steadily as the contrast of the target increases, and this reduction is statistically significant, $\chi^2(4) = 25.38$, $p < 0.001$. We interpret this outcome to indicate that voluntary binocular searching movements are always present in the discrimination condition, but they are modulated by the visibility of the stimulus. On the other hand, the frequency of monocular microsaccades reaches a peak around the discrimination threshold (62.5% correct, between the third and fourth contrast level) and then decreases at the highest visibility for the dominant, $\chi^2(1) = 11.65$, $p < 0.001$, but not for the nondominant eye, $\chi^2(1) = 2.85$, $p = 0.092$. This significant variation in the frequency of monocular microsaccades might imply a role for these

movements in the process of making fine discrimination (see also Figures D2 and D3).

Discussion

Microsaccades have received considerable recent interest despite controversy about the functions that they are supposed to fulfill (Collewijn & Kowler, 2008; Ditchburn & Foley-Fisher, 1967; Ditchburn & Ginsborg, 1953; Rolfs, 2009). *Binocular* microsaccades have been shown to counteract visual fading (Martinez-Conde et al., 2006; McCamy et al., 2012), to preferentially scan small and informative visual regions (Otero-Millan, Troncoso, Macknik, Serrano-Pedraza, & Martinez-Conde, 2008), to trigger perceptual transitions in bistable illusions (van Dam & van Ee, 2005; but see van Dam & van Ee, 2006), and during monocular viewing, to improve visual discrimination (McCamy et al., 2012). Thus, binocular microsaccades are thought to be either reflexive or fixational, when the subjects attempt to maintain steady fixation on a target, or exploratory, when subjects are free to move their eyes. Our work gives new insights about this behavior.

Taken together, our results suggest that monocular microsaccades might be aimed at reducing small horizontal and vertical vergence errors, potentially reducing binocular disparity, facilitating binocular summation (Heravian-Shandiz, Douthwaite, & Jenkins, 1993), and thereby the success of discrimination. The increased frequency of monocular microsaccades near the threshold contrast level also suggests that they might contribute to fine visual discrimination by correcting the gaze position toward the preferred retinal locus of fixation of each or a preferred eye. These two hypotheses are not mutually exclusive and might contribute together to facilitate fine spatial judgments. Finally, the increased frequency of monocular microsaccades that we observe near the threshold-contrast level is in agreement with recent work by Ko et al. (2010) and Poletti et al. (2013), who suggested a dynamic corrective role for microsaccades (during *monocular* viewing and recording, so without being able to distinguish between monocular and binocular microsaccades). It would be interesting to extend the present finding in the experimental paradigm used by Poletti et al. (2013) by undertaking binocular viewing and recording.

Similar to our observation that monocular microsaccades occur less frequently during discrimination than during fixation, Ko et al. (2010) reported that the mean rate of microsaccades was lower when subjects performed a high-acuity *monocular* needle-threading task compared to fixation on a visual target. Interestingly, Ko et al. (2010) also observed that the microsaccade rate dropped when the distance between the tip of the thread and the needle was smaller than 5′ (i.e., when it may be assumed there was no need to relocate the image within fovea). This observation might be similar to our finding that the frequency of monocular microsaccades decreases when the C target is most highly visible. Although Ko et al. (2010) stressed the need to distinguish between exploratory and fixational microsaccades, they recognized the difficulty in discriminating between them practically.

We find that monocular microsaccades occurred in all tested subjects, share a common generator with their binocular microsaccade and saccadic counterparts and cannot be accounted for by either saccadic errors or as recording noise. As for binocular microsaccades, they seem to be modulated by the visual demand of the task, but in a very different way. The binocular microsaccade rate during C discrimination remains high when subjects perform around chance level, and decreases steadily with visibility. This is in agreement with an exploratory role of binocular microsaccades during discrimination, where a foveated C will require fewer microsaccades to confirm the gap position at high compared to low visibility.

On the other hand, the rate of monocular microsaccades in the sighting-dominant eye reaches a peak around the visibility threshold, before dropping at high C visibility. Although the whole image of the C is projected onto the fovea, a small correction of one eye's line of sight within the order of a few minutes of arc might provide a substantial benefit for discrimination within the foveola. The frequency of monocular microsaccades thus depends on the precision of intended fixation (see also Poletti & Rucci, 2010) making them likely to be involved in the precise relocation of the line of sight *and/or* the reduction of binocular disparity. The amplitude range of the monocular microsaccades (from 8′ to 20′) supports the hypothesis of single or dual, but not conjugated, monocular correction mechanisms that could operate to move gaze to the preferred locus of fixation, believed to be with a standard deviation of only 3.4′ (Putnam et al., 2005). Uniocularly controlled movements, occurring more frequently at the threshold level might also, but not exclusively, serve to correct for fixation disparity, thereby locally facilitating binocular summation and visual discrimination. Further work is necessary to disentangle the roles of vergence and/or position-correcting strategies that employ monocular microsaccades generated by nonconverging monocular premotor signals.

Finally, the apparent unilateral control of each eye at the microsaccadic level provides a new challenge to Hering's law of equal innervation (Hering, 1977), in agreement with neurophysiological studies (Hafed, Goffart, & Krauzlis, 2009; Van Gisbergen, Robinson,

& Gielen, 1981; Zhou & King, 1998), and in the context of larger eye movements (Enright, 1996; King, 2011). Evidence indicates that each individual muscle is driven by independent populations of neurons (Dell'Osso, 1994), and that agonist muscles, although usually coordinated, may function independently if the task requires. In agreement with this view, our results offer new clues as well as a new paradigm to reevaluate Hering's law of equal innervation.

Keywords: microsaccade, monocular microsaccade, fixational eye movements, oculomotor control, visual perception

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Corresponding author: Josselin Gautier.
Email: josselin.gautier@davalorsalud.com.
Address: Davalor Research Center, Universitat Politècnica de Catalunya, Barcelona, Spain.

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Appendix A. Supplementary Methods

Eye tracking (continued)

Before each session, nine fixation dots positioned in a 3×3 grid were presented successively for calibration and validation. Calibration and validation were repeated if the deviation from the central calibration dot for either of the two eyes during validation exceeded 0.25°. Background illumination during calibration and validation was similar to that during the experiment to minimize subsequent variations in pupil size. During the experiment, we did not observe any consistent changes in pupil size that occurred in synchrony with the subjects' eye movements (see Appendix C) as observed by Kimmel et al. (2012) for saccades having an amplitude on the order of 1°.

Eye movement data were recorded for the 100 ms before each stimulus presentation as well as the 2000-ms duration of each trial. We observed idiosyncratic slow drift in the recorded DC position of each eye from trial to trial. Although this drift made it difficult to determine the absolute eye position with respect to the experimental targets, it did not interfere with the detection and characterization of microsaccades. The eye-tracking software of the EyeLink was configured to track the center-of-mass of the pupil (*centroid* mode) and the embedded saccade detection filter was set to *high* sensitivity, as advised by the manufacturer for the identification of small saccades.

Stimuli and procedures

The experiment included a fixation and a discrimination condition, run in separate blocks of 100 consecutive trials. In the fixation condition, subjects were instructed to maintain fixation at the center of a light cross with a Weber contrast of 9.8% that was presented at the middle of the display screen for 2 s. This relatively low contrast was chosen to ensure adequate visibility while minimizing possible pupillary responses at stimulus onset. (Ukwade & Bedell, 1993) reported previously that fixation stability is unaffected by the Michaelson contrast of a fixation target in the range from 7% to 84%. After each 2-s presentation of the fixation cross, the subjects viewed a uniform gray background until they pressed a button to begin the next trial.

In the discrimination condition, subjects were instructed to discriminate the position of the gap in a 40×40 min arc square letter C, presented at the center of the display screen. The letters (Cs) were darker than the background and presented with one of five different Weber contrasts, ranging from −1.06% to −2.1% (see also the axis of Figure 5). Both the contrast of the C and the location of the gap (right, left, up, or down) varied randomly from trial to trial. After each 2-s presentation of a C, a uniform gray background remained visible until the observer reported the location of the gap using a four-button response box. Observers were instructed to press any button if the position of the gap was not perceived. The button press initiated the next trial.

Eye-movement data analysis

A microsaccade detection algorithm adapted from (Engbert & Mergenthaler, 2006) was used to identify fixational saccades. A velocity threshold, $\eta_{x,y}$, was set to a multiple ($\lambda = 6$) of the median eye velocity for each trial for each subject. A minimum duration, τ of 12 ms (six samples) was chosen to avoid false identifications of microsaccades from brief intervals of noise.

As described by (Kimmel et al., 2012), we observed that noise in the eye-position signal sometimes broke

a single microsaccade into two or more physiologically improbable "sub-saccades." Thus, we chose to fuse any two microsaccades that were separated by less than 10 ms (i.e., fewer than five samples) into a single movement. To eliminate possible artifacts associated with the onset and offset of blinks, 200 ms of the recorded eye-position signal was removed before and after each blink that was identified by the EyeLink software.

Appendix B. Robustness of monocular microsaccade detection

To validate the robustness of the detection method for monocular microsaccades and the three criteria used (λ, τ, and a minimum temporal overlap for binocularity set to one sample, as in the literature), we performed a separate velocity noise analysis. First, we asked whether a less conservative minimum duration threshold than the one we use (12 ms, compared to the typical value of 6–8 ms in the literature) would be more appropriate. We transposed the method of Engbert based on surrogate data generation and signal-to-noise ratio analysis (Engbert & Mergenthaler, 2006) to see if we could estimate the optimal minimal duration instead of optimal velocity threshold. The theoretical best value ($\lambda < 2$), obtained with the original method from Engbert and Mergenthaler (2006) with binocular detection, is implausible, making this method not appropriate in the identification of a putative optimal velocity threshold. Still, by using the original long minimal duration (τ of 12 ms), the putative optimal signal-to-noise ratio was obtained with a velocity threshold of $\lambda = 3.5$ which is again implausible. Plots of microsaccades (traces, main sequence) show that this velocity criterion includes the identification of a lot of noisy high velocity drift as microsaccades. We hypothesized that a second method, based on surrogate data, to account for nonlinearity within variables (i.e., between each eye velocity), proposed by Prichard and Theiler (1994) might be more appropriate in this case than their original work (Theiler, Eubank, Longtin, Galdrikian, & Farmer, 1992). This updated method should preserve the linear autocorrelations and cross correlations between the eyes' velocity series but, in practice, lead to an overdetection of monocular microsaccades from these surrogate data.

Thus, we tested a third method to validate the robustness of the detection of monocular microsaccades and potentially find an optimal velocity criterion. Instead of generating surrogate data in the velocity domain, we added velocity noise to one or both eye's velocity components. We add to the velocity component on each trial the velocity drift + noise elements obtained from the velocity components from the next two consecutive trials. Hence, the independence between eyes is respected, while the velocity component comprising drift and noise is veridical because it is identified, extracted, and then added to the same eye. By adding different amounts of multiplicative noise (from a factor of unity to 6) to one or both eyes and then performing microsaccade detection, the detection rate is clearly not affected in the range of velocity threshold we used; there is no overdetection of monocular microsaccades with an increase in the noise factor compared to without noise.

Finally, we tested if the third, temporal overlap criterion used to identify the complementary binocular microsaccades (one minimum time sample in common between two microsaccades) could play a role in potentially overdetecting monocular microsaccades. We determined that by increasing the minimum temporal overlap from one sample (2 ms) to two up to five, no specific change in either the monocular or binocular microsaccade detection was found.

Taken together, these results suggest that the set of detection thresholds (λ, τ, and a minimum temporal overlap for binocularity) used in our study to identify both monocular and binocular microsaccades is appropriate. These simulation results also rule out a putative hypothesis that the identified monocular microsaccades are due to a local independent nonlinearity or velocity noise in the signal of one or both eyes.

Appendix C. Pupil change interaction with monocular microsaccades

Kimmel et al. (2012) reported that pupil size oscillates in phase with the position change detected by the eye tracker optical system, and potentially represents an artifactual interaction between pupil size and eye position: "[W]e found a significant position-pupil interaction only for the horizontal dimension in the optical system, whereas position in the vertical dimension and both dimensions in the coil system were not influenced by changes in pupil sizes" (p. 14; Note: The pupil is measured with the optical system).

Choe et al. (2016) reported the degree of correlation between the change of pupil area (PA) and the change of gaze position (GP) at two time scales:

"The degree of PA-GP correlation varied greatly across observers and between eyes in a given individual as well.

[W]e applied the same correction procedure to the Gaze Position (GP) measurements separately for different eyes, for individual observers and for individual runs. The consequences of correction for the PA-confounded errors were replicated. The variances of GP that could be accounted for the by the PA regressor (55.8% ± 28.3% and 33.1% ± 26.4% for the left and right eye, respectively) were comparable to those derived from experiment 1" (p. 9).

Nevertheless, Choe et al. (2016) reported "a negligible influence of the correction procedure on (authors note: binocular) microsaccade detection" (p. 9).

We measured the relationship between the change in horizontal gaze positions of the left eye or both eyes during detected monocular microsaccades and the corresponding pupil area change during this time interval. To do so, we performed linear regression over a much shorter time scale (approx. 10–15 ms) than over the "short-interval" of 13.2 s reported by Choe et al. (2016). (Note: To avoid any delay of the eye position measurement relatively to pupil area due to the 5×5 spatial filter from the original Engbert & Mergenthaler (2006) detection method, we switched the filter off to measure correlation over time). The regression (R^2) and corresponding p-values were determined only with a minimum number of 10 observations (i.e., in cases where each eye made more than 10 monocular microsaccades), which include 23 of the 32 experimental runs (2 eyes × 2 sessions × 8 subjects). The variance of monocular microsaccade horizontal amplitude that could be accounted for by the PA regressor (30.5% ± 27.3% and 19.3% ± 28.5% for the left and right eye, respectively; mean ± *SD* across observers and sessions) was low and similar to that for the vertical change in eye position (33.6% ± 28.9% and 22.5% ± 18.0%).

In order to take into account the changes in both horizontal and vertical components that potentially could be explained by a change in PA, we constructed a multiple linear regression model of PA change with saccade horizontal and vertical components as multiple regressors. Again, the variance of the PA change that could be accounted for by a change in monocular microsaccades horizontal and vertical components varied considerably between eyes and individuals (from 0.6% to 93.1%, with mean values of 49.8 ± 27.3% and 31.4 ± 24.5% for the left and right eye, respectively) but remains low. However, the correlation between PA changes and eye-position changes was significant ($p < 0.05$) for 12 of the 23 eyes × sessions × subjects.

If a change in PA does not account well for a change in the horizontal or both horizontal and vertical variation during monocular microsaccades, then one might wonder whether the first-order derivative (i.e., peak velocity) between eye movement and PA might yield a better explanation of any potential correlation. The variance in eye velocity accounted for by regression varies a lot from subject to subject and between the two eyes of the same subject (from 0.1% to 94.6%) but on average remains low (29.3% ± 27.4% and 24.9% ± 36.24% for the left and right eyes, respectively).

Finally, we considered whether the *peak velocity of the PA* during a monocular microsaccade better correlated with the peak velocity of the identified monocular microsaccade rather than the pupil area change measure at the same time of that peak (i.e., by relaxing the constraint that both peak velocities had to occur at the same time). By regressing the pupil peak velocity with the peak velocity of the monocular microsaccades, we found that the variance accounted for is even lower (ranging from 0.0% to 92.2%, mean values 18.6 ± 26.3% and 12.91 ± 26.99% for the left and right eye, across observers and sessions). We replicated these observations by pooling the regression for each eye of each observer across different sessions.

Finally, by plotting the velocities of PA with eye position during monocular microsaccades we found cases where the PA variation seemed to occur before a change in eye position. If a change in PA produces an artifactual change in measured eye position—leading to erroneously detected monocular microsaccades—then we should expect in a majority of cases the change of pupil area to either lead or occur concurrently with a change in eye position. By measuring the phase between the peak velocities of PA change and monocular microsaccade, we determined the number of instances in which the PA change led versus lagged monocular microsaccade peak velocity. Because the number of identified monocular microsaccades per session for each eye of each subject is close to the minimum value (5) required for χ^2 analysis, we pooled the data across multiple sessions for each observer.

We observed that in the majority of monocular microsaccade events (per eye per subject), the peak velocity of PA leads to the peak velocity of microsaccades. However, for only half of the observers, we observed a significant number of instances in which the velocity of PA change led the peak velocity of monocular microsaccades in both eyes (after Bonferroni correction). The eye tracker manufacturer confirmed that the pupil and eye position samples are output at the same time, and that this applies for the three possible sample-to-sample filtering values that can be applied, eliminating a putative effect of the eye tracker.

Thus, we conclude that there is minimal evidence that changes in PA occurred before the identification of monocular microsaccade in our data. It therefore appears very unlikely that pupil area changes can account for the numerous observations of monocular microsaccades according to our various regression analyses.

Appendix D. Microsaccade rate (1) per τ, (2) per subject, and (3) per visibility step on successful discrimination trials

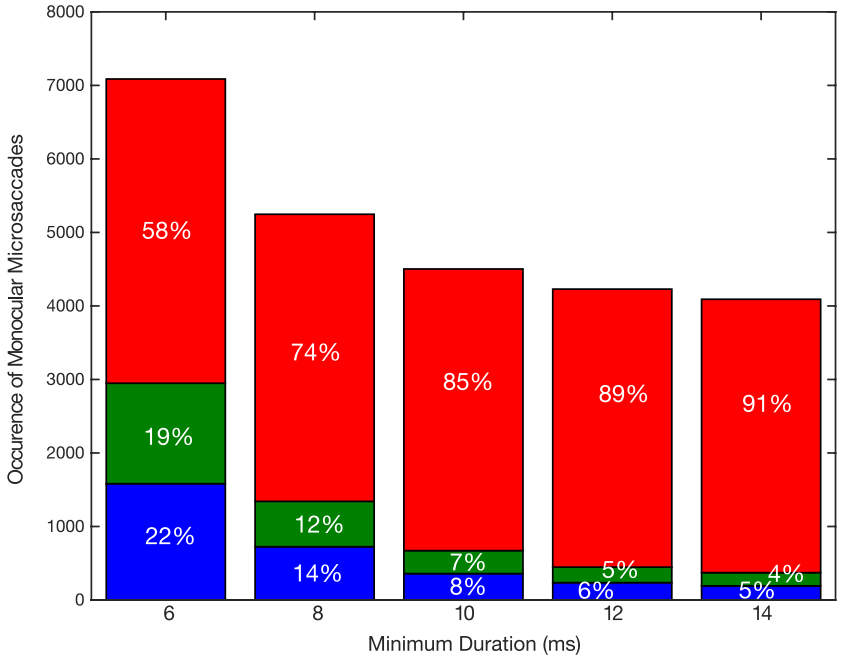

Figure D1. Impact of the minimum duration criterion on the numbers and proportions of microsaccades. Left- (blue) and right- (green) eye monocular and binocular (red) microsaccade occurrence and ratio are shown for different minimum duration thresholds. Although a minimum duration threshold of 6–8 ms yields a similar proportion (approx. 40%) of monocular microsaccades as in some previous studies (see references in the main text), we adopted a more conservative threshold of 12 ms to cope with potential velocity noise from the video eyetracker.

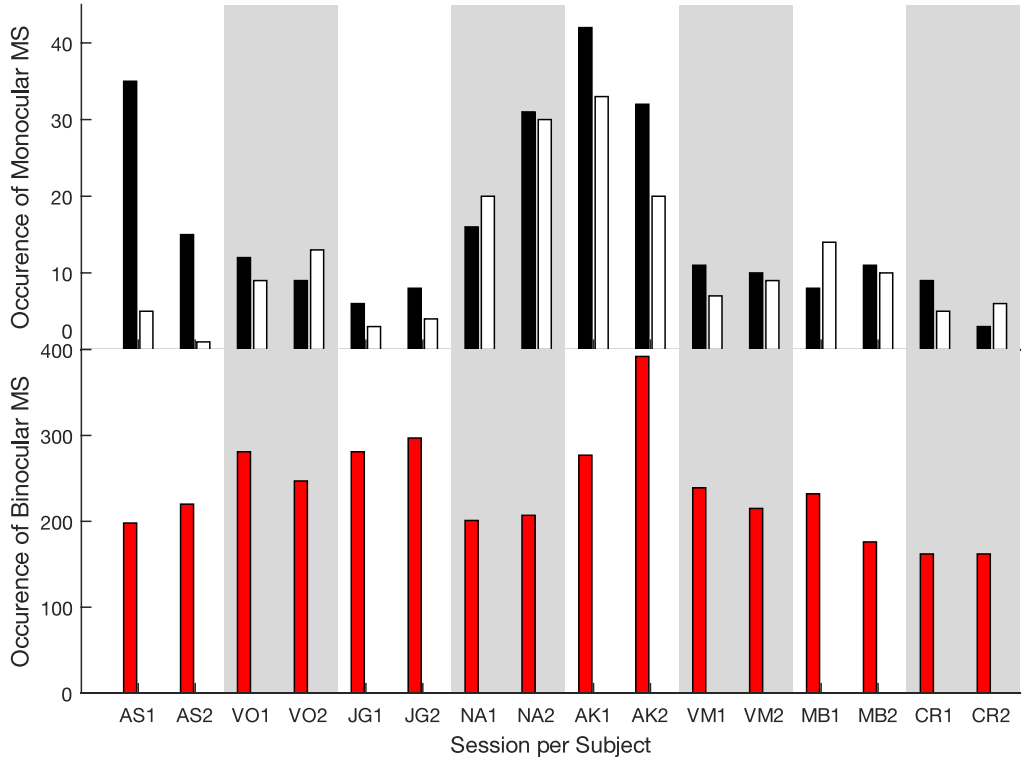

Figure D2. Monocular (top) and binocular (red, bottom) microsaccade occurrence for the dominant (black) and nondominant eye (white) of each subject and experimental session (two sessions per subject). White and gray vertical backgrounds strips group the two sessions for each subject.

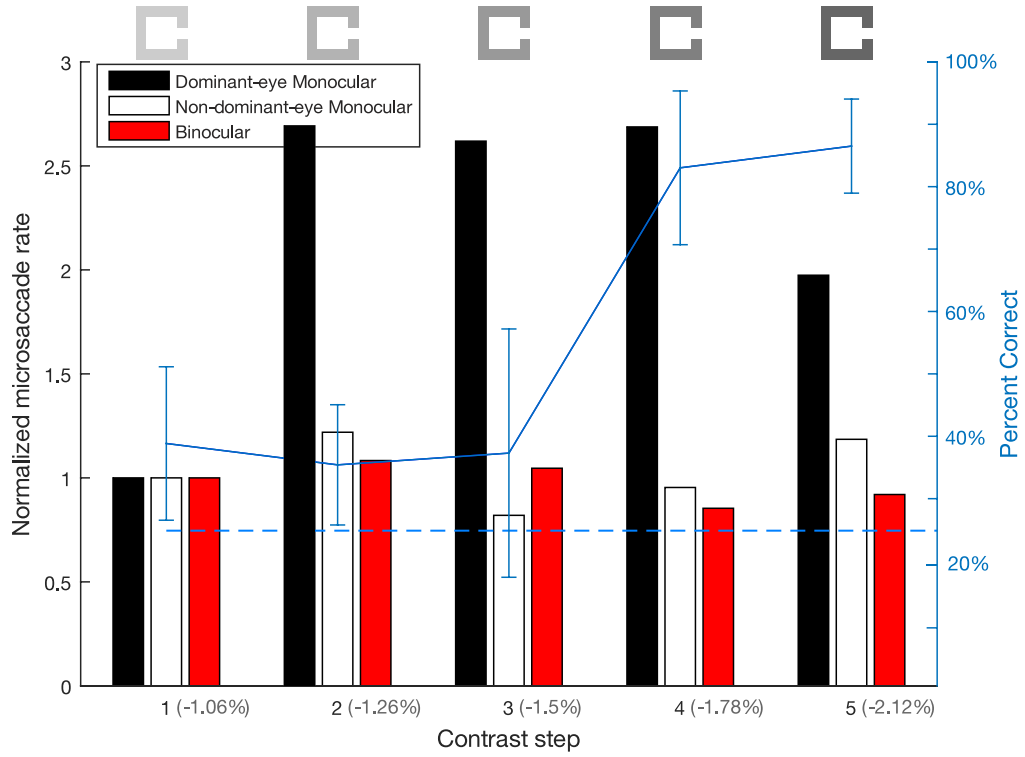

Figure D3. Same as text Figure 5 but showing the normalized binocular and monocular microsaccade rates on *successful* discrimination trials only.